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CD36 Gene Promoter Polymorphisms Are Associated With Low Density Lipoprotein-Cholesterol in Normal Twins and After a Low-Calorie Diet in Obese Subjects

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Common polymorphisms of the *CD36* fatty acid transporter gene have been associated with lipid metabolism and cardiovascular disease. Association of a *CD36* promoter single nucleotide polymorphism genotype with anthropometry and serum lipids was investigated in normal subjects, and in obese subjects during an 8-week low calorie diet and 6-month weight-maintenance period. 2728 normal female Twins UK subjects (mean body mass index 24.8 ± 4.4 kg/m^2; age 47.3 ± 12.5 y) and 183 obese male and female Spanish subjects (mean body mass index 30.6 ± 3.0 kg/m^2; age 35.0 ± 5.0 y) were genotyped for the *CD36* -22674 *T/C* (rs2151916) promoter single nucleotide polymorphism. In the Twins UK full cohort, the *C*-allele was associated with lower low density lipoprotein-cholesterol (p = .02, N = 2396). No associations were found in the obese Spanish subjects at baseline, but 6 months after the end of the low-calorie diet, the *C*-allele was associated with lower total- (p = .03) and low density lipoprotein-cholesterol (p = .01) and higher high density lipoprotein-cholesterol (p = .01). Intake of saturated fatty acids was lower in carriers of the *C*-allele at baseline, but not significantly so (p = .11). However, 6 months after the end of the low-calorie diet, elements of the lipid profile were correlated with saturated fatty acid intake: total cholesterol r = .21, p = .060; low density lipoprotein-cholesterol: r = .25, p = .043; high density lipoprotein-cholesterol: r= –.26, p = .007. *CD36* promoter SNP allele –22674*C* is therefore associated with lower serum low-density lipoprotein-cholesterol in normal female twins and with improved lipid profile during weight loss and maintenance in obese subjects.

Keywords: *CD36* promoter, SNP association study, LDL-cholesterol, low calorie diet (LCD)

CD36 is an integral membrane glycoprotein with broad ligand-binding specificity and a variety of functions in lipid transport, immune regulation, hemostasis, signal transduction, adhesion, angiogenesis and atherosclerosis (Greenwalt et al., 1992; Nicholson & Hajjar, 2004; Silverstein & Febbraio, 2000). The protein facilitates the membrane transport of long chain fatty acids into muscle and adipose tissue and CD36 deficiency is associated with a reduction in fatty acid uptake (Tanaka et al., 2001). Consistent with this role, the receptor is prevalent in tissues with a high metabolic capacity for long-chain fatty acids (LCFAs) (Abumrad et al., 1993): the proximal intestine, where most lipid absorption occurs (Chen et al., 2001), adipose tissue (Abumrad et al., 1993), where fatty acid is stored as neutral lipids, in the heart (Luiken et al., 1999), which relies heavily on fatty acid oxidation and in skeletal muscles with predominantly oxidative fibres (Bonen et al., 1999). CD36 is also important for both secretion and clearance of intestinal lipoproteins (Drover et al., 2005). In an interesting development, CD36 appears to be a plausible candidate for the spontaneous attraction for dietary lipids shown by mice and rats (Laugerette et al., 2005) owing to its high affinity for LCFAs and location in lingual papillae. CD36 gene inactivation abolished the rodents' normal preference for LCFA-enriched solutions, raising the possibility that an alteration in lingual fat perception may be linked to changes in dietary preference (Abumrad, 2005).

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Consistent with its role in fatty acid transport, there is strong evidence for regulation of CD36 expression by dietary and metabolic factors. Expression is upregulated by dietary fat in adipose tissue (Nisoli et al., 2000) and heart muscle (Greenwalt et al., 1995) and altered in obesity and type 2 diabetes (Bonen et al., 2006).

Genetic variability at the *CD36* locus may be a determinant of plasma levels of non-esterified fatty acids (NEFA). Ma et al. (2004) typed 21 polymorphic markers spanning the *CD36* gene in 585 nondiabetic Caucasians and found an association with NEFA and SNP-33137 *A/G* (p = .008) in the promoter region flanking exon 1a, in 217 men. They also identified a potentially functional common variant at –22674 *T/C* in strong LD with a 5-SNP haplotype associated with 31% higher NEFA (p = .0002) and 20% higher triglycerides (p = .025) than in noncarriers, but they did not test associations with this SNP directly. We have genotyped –22674 *T/C* (rs2151916) in a more powerful study of normal subjects than Ma et al. and in a sample of obese subjects of comparable size to their gender groups. In 2728 normal female Caucasian subjects from the Twins UK cohort, we tested genotype associations with waist circumference, triglyceride, total cholesterol and high-density lipoprotein (HDL) cholesterol, for which Ma et al. observed nonsignificant trends, plus additional anthropometric variables. We tested association with the same variables in 183 healthy overweight or obese Spanish subjects at baseline, after a 8-week low-calorie diet (LCD) and the end of a 6-month weight-maintenance period. Finally, to investigate possible influence on preference for fat, –22674 *T/C* association with components of dietary intake was tested before and after the LCD.

Materials and Methods

Subjects

The Twins UK (St Thomas' UK Adult Twin Registry) comprises unselected, mostly female volunteers ascertained from the general population through national media campaigns in the United Kingdom (Spector & Williams, 2006). The study cohort comprised 2728 randomly selected subjects (826 monozygous (MZ) and 1902 dizygous (DZ). Means and ranges of quantitative phenotypes in Twins UK were similar to an age-matched sample from the general population in the UK (Andrew et al., 2001). Informed consent was obtained from participants before they entered the study and approved by the local research ethics committee. The Spanish Caucasian subjects recruited to participate in the dietary intervention study comprised a group of 183 (88 male/95 female) with excess body weight. The BMI range at entry was 27–40 kg/m^2, following the SEEDO criteria (Salas-Salvadó et al., 2007). Initial screening evaluations included a medical history, physical examination and fasting blood profile, to exclude subjects with clinical disorders. Other exclusion criteria were weight change > 3 kg within the 3 months before the start of the study, drug treatment, pregnancy, previous surgically or drug-treated obesity, alcohol- or drug-abuse. After a detailed explanation of the study, a written informed acceptance was obtained from all participants before the beginning of the trial, in agreement with the Helsinki Declaration. This consent document and the study protocol were previously approved by the Ethical Committee of the University Clinic of Navarra (Ref. 54/2006). The characteristics of subjects in both study cohorts are shown in Tables 1 and 2.

LCD Study Design in Spanish Obese Subjects

The 183 subjects were enrolled in an 8-week dietary intervention program with a balanced LCD prescribed according to a food exchange system described elsewhere (Thorsdottir et al., 2007), designed to supply 55% of energy as carbohydrates, 15% as proteins and 30% as fat. Weight loss was measured weekly and the dietary compliance was monitored by means of 3-d weighted food diary records (2 weekdays and 1 weekend day; Xinying et al., 2004), performed during the week before the beginning and the week before the end of the nutritional interventional trial. Basal metabolic rate (BMR) was estimated by applying Harris-Benedict equations, using a correction factor due to the overweight status of the subject (Cankayali et al., 2004). To estimate the total energy expenditure (EE), the BMR was corrected by the physical activity level based on World Health Organization (WHO) criteria (World Health Organization, 2000). The calorie restriction was set up at 30% lower than the total estimated EE.

After finishing the dietary intervention, volunteers were given dietary guidelines to maintain the weight-loss, but without calorie-restriction or specific

Table 1

Twins UK: Characteristics of Subjects

	Total		BMI≥27 kg/m^2	
Variable	*n*	*Mean ± SD*	*n*	*Mean ± SD*
Age, years[a]	2728	47.3, 12.5	654	49.8, 11.7
Obesity-related variables:				
BMI, kg/m^2	2712	24.8, 4.4	654	30.8, 4.0
Weight, kg	2713	65.4, 11.9	654	80.2, 11.5
Waist, cm	2662	78.4, 10.2	643	90.6, 9.5
Total fat, kg	2672	23.5, 8.8	644	34.5, 8.0
Total fat, %	2632	35.6, 8.0	636	43.6, 5.8
Central fat, kg	2630	1.3, 0.7	635	2.2, 0.7
Central fat, %	2630	31.2, 11.5	635	42.2, 8.1
Lipid profile[b]:				
Triglyceride, mmol/l	2443	1.3, 0.8	602	1.6, 0.9
Cholesterol	2575	5.6, 1.3	622	5.9, 1.3
HDL-c	2583	1.5, 0.4	624	1.4, 0.4
LDL-c	2436	3.5, 1.2	597	3.8, 1.1

Note: [a]Number of subjects with genotype data: Total: 715 MZ, 1642 DZ; BMI≥27: 155 MZ, 499 DZ.
[b]Subjects using lipid-lowering agents were excluded.

follow-up instructions. A total of 84 subjects returned 6 months after the end of the nutritional intervention period and were measured for weight maintenance and potential changes in anthropometry and body composition measurements, as well as biochemical markers. The physical activity pattern remained unchanged in all the stages, which was assessed and controlled by a trained specialist, based on a validated physical activity questionnaire for overweight individuals (Martínez-González et al., 2005).

Zygosity, Body Composition and Biochemical Analyses

Zygosity in Twins UK subjects was determined by standardized questionnaire and confirmed by DNA fingerprinting. Body weight, height, waist circumference, body composition and blood pressure were measured in the twin and Spanish subjects as previously described (Goyenechea et al., 2007; Jamshidi et al., 2006). Total and central body fat measurements of the twins were obtained by dual emission X-ray absorption (DEXA) body composition scans (Hologic QDR-2000, Vertec, Waltham, MA). Blood samples for analyses were drawn after a minimum 8-h overnight fast and serum was stored at –45 °C until analyzed. Twin serum lipids were analyzed using the Cobas Fara and Spanish samples using the Cobas Mira automatized systems (Roche, Basel, Switzerland). A colorimetric enzymatic method was used to determine total cholesterol, triglyceride and HDL cholesterol levels. The latter was measured after precipitation from chylomicron, low density liporotein (LDL) and very low density liporotein (VLDL) particles by magnesium and dextran sulphate. The Friedewald equation (Friedewald et al., 1972) was used to calculate LDL-cholesterol levels in subjects with triglycerides ≥ 4·52 mmol.L^{-1}.

Genotyping

The SNP –22674 *T/C* was genotyped by Pyrosequencing (Biotage, Uppsala, Sweden). Genotyping accuracy, as assessed by inclusion of duplicates (pairs of MZ twins) in the arrays, was 98% and negative controls (water blanks) were included on each plate. Genotyping success rates varied between 94.3% and 96.5%. Primers and PCR conditions for SNP genotyping by Pyrosequencing are available upon request.

Statistical Analysis

A chi-square (χ^2) test was used to evaluate Hardy-Weinberg equilibrium in both samples. In Twins UK, one twin of each pair was chosen at random to prevent inflated significance and preliminary analyses were performed using STATA 8 (StataCorp, College Station, Texas). Where needed, phenotypic variables in Twins UK were log transformed to obtain better approximations of the normal distribution prior to analysis. For association analysis in the Twins UK cohort, MZ and DZ twins were analyzed together using Generalized Estimating Equations (GEE; Trégouët et al., 1997). This allows for the relatedness between twins and yields unbiased standard errors and *p* values. For each single nucleotide polymorphism (SNP), we first performed a 2-df overall test of genotypic association. In the presence of a significant association, dominant and recessive models (all 1-df) were further tested to find the best mode of inheritance. Age and menopausal status were included as covariates in the models.

In the Spanish subjects statistical analyses were carried out using the SPSS 13.0 program for Windows XP (Microsoft, USA). A two-tailed *p* value less than .05 was chosen as the level of statistical significance. As several anthropometrical (e.g., body weight, BMI, fat mass) and clinical (e.g., glucose and insulin) variables are not completely independent, the Bonferroni test for multiple comparison was considered too conservative for the current analysis. Spearman's correlation coefficient was calculated to determine relationships between variables. Analysis of variance (ANOVA), student's t-test (for dominant model) and ANOVA for repeated measures were used to analyse differences with respect to genotypes on weight-loss and maintenance processes. Lipid profile variables were adjusted for BMI by the residuals method.

Table 2

Baseline Anthopomentrical and Fasting Lipid Profile Characteristics of Obese Spanish Subjects

Variable	Males Mean ± *SD*	Females Mean ± *SD*
n	95	88
Age, y	35.6, 6.6	34.6, 6.2
Obesity-related variables:		
BMI, kg/m^2	31.1, 2.6	31.3, 3.3
Weight, kg	95.7, 10.7	84.5, 12.6
Waist, cm	100.8, 7.6	92.2, 8.9
Total fat, kg	27.8, 6.9	33.6, 7.7
Total fat, %	28.9, 5.8	39.5, 6.1
Lipid profile[a]:		
Triglyceride, mmol/l	1.2, 0.5	1.2, 0.5
Cholesterol, mmol/l	5.4, 1.0	5.2, 0.9
HDL-cholesterol, mmol/l	1.2, 0.2	1.4, 0.4
LDL-cholesterol, mmol/l	3.6, 0.9	3.2, 0.8

Note: [a]Lipid profile variables adjusted for BMI.

Results

Genotype Association With LDL-Cholesterol in Twins UK Subjects

We first genotyped the *CD36* promoter SNP -22674 *T/C* in the Twins UK cohort. The allele and genotype frequencies were as follows: *T* = 0.63; *C* = 0.37; *TT* 38.9%; *TC* 48.5%; *CC* 12.6%. Genotype frequencies corresponded to Hardy-Weinberg proportions. Table 3 shows tests of association of phenotypic variables with genotypes in the full cohort. Mean levels of LDL-cholesterol were significantly lower in rare allele *C* homozygotes compared to common allele *T* carriers and the association accounted for 0.21% of variance.

Table 3

Association of *CD36* Promoter SNP –22674 *T/C* Genotype with Metabolic Variables in All Twins UK Subjects

Variables[a]	No. 11/12/22	Genotype 11 Mean ± *SD*	12 Mean ± *SD*	22 Mean ± *SD*	GEE *p*-value[b]
BMI, kg/m^2	1043/1294/330	24.8, 4.5	24.7, 4.2	25.0, 4.8	0.71
Weight, kg	1043/1295/330	65.5, 11.9	65.1, 11.5	65.8, 13.0	0.93
Waist, cm	1023/1272/324	78.3, 10.4	78.4, 9.9	78.7, 10.6	0.83
Total fat, kg	1026/1283/320	23.4, 9.0	23.4, 8.6	23.7, 9.1	0.92
Total fat, %	1009/1267/313	35.5, 8.2	35.6, 8.0	35.7, 7.7	0.96
Central fat, kg	1017/1275/314	1.3, 0.8	1.3, 0.7	1.3, 0.7	0.85
Central fat, %	1017/1275/314	31.0, 11.8	31.3, 11.5	31.4, 10.6	0.9
Triglyceride, mmol/l	945/1181/297	1.2, 0.8	1.3, 0.8	1.3, 0.8	0.3
Cholesterol, mmol/l	986/1239/309	5.5, 1.3	5.6, 1.3	5.5, 1.2	0.14
HDL-cholesterol, mmol/l	984/1247/309	1.5, 0.4	1.5, 0.4	1.6, 0.4	0.76
LDL-cholesterol, mmol/l	932/1169/295	3.5, 1.2	3.5, 1.1	3.4, 1.1	0.04

Note: [a]All variables were log transformed except total fat % and central fat %.

[b]The GEE model for all variables was adjusted by age and menopausal status. The GEE model for triglyceride, cholesterol, HDL and LDL was adjusted for age, menopausal status, fasting status and BMI. *p*-values based on a co-dominant model.

Absence of Genotype Associations in Overweight and Obese Twins UK Subjects

For comparison with Spanish overweight and obese subjects, we examined association of –22674 *T/C* with biochemical and physiological variables in Twins UK subjects with BMI ≥ 27 kg/m^2. Table 1 shows the clinical and biochemical characteristics of the Twins UK overweight subgroup. There were 654 subjects with BMI ≥ 27 kg/m^2 (mean 30.8 ± 4.0 kg/m^2). We found no significant associations between genotypes and phenotypic variables in these overweight/obese twin subjects (data not shown).

Genotype Associations With Metabolic Variables in Obese Spanish Subjects

We began our investigation of the Spanish overweight or obese subjects taking the low-calorie diet (LCD) by genotyping –22674 *T/C* in all subjects (N = 183, mean age 35.0 ± 5.0 y). The allele and genotype frequencies were as follows: T = 0.62 C = 0.38; *TT* 37.1%; *TC* 50.3%; *CC* 12.6%. Genotype frequencies corresponded to Hardy-Weinberg proportions and were similar to those found in the Twins UK cohort. We tested association of anthropometry, body composition and fasting serum lipids with –22674 *T/C* genotypes at three time points: baseline, at the end of the 8-week low-calorie diet (LCD) and at the end of a 6-month weight maintenance period. As we observed in the overweight/obese twin subjects (N = 654), at baseline there were no significant differences in anthropometry, body composition and fasting serum lipids with respect to genotype (data not shown).

Genotype Associations With Serum Lipids After LCD and Weight Maintenance

The repeated measures ANOVA showed no time-genotype interactions for any of the clinical variables, which allowed us to analyse each effect separately. As was expected, the time evolution effect was significantly observed for most of the variables (Table 4). The overall genotype effect was observed for total- and LDL-cholesterol (Table 4). The rest of the clinical variables did not differ with respect to genotype. There was a significant fall in total cholesterol between baseline and the 8-week LCD endpoint and between endpoint and 6 months of weight maintenance associated with the *C*-allele ($p < .05$). Then, after 6 months the *C*-allele was significantly associated with lower total- and LDL-cholesterol and higher HDL-cholesterol, based on a co-dominant model. No differences were observed for the other anthropometric and clinical variables (Table 4).

Genotype Associations With Dietary Intake After LCD and Weight Maintenance

Table 5 shows association of elements of dietary intake with genotypes at baseline and at the end of the 8-week low-calorie diet. Intake of saturated fatty acids (SFA) was lower in carriers of the *C*-allele at baseline, but not significantly so under the codominant model. However, at 6 months after the LCD, elements of the lipid profile were correlated with SFA intake: total cholesterol r = .21, p = .060; LDL-cholesterol: r = .25, p = .043; HDL-cholesterol: r = –.26, p = .007.

Discussion

We investigated association of the genotypes of a potentially functional *CD36* promoter SNP –22674 *T/C* with anthropometrics and lipid profile in 2728 normal British female twins and 183 obese male and female Spanish subjects, and during an 8-week LCD and 6-month maintenance period in the obese group. Association of –22674 allele *C* with significantly lower LDL-cholesterol was found in 2396 Twins UK women, but no significant association could be detected in a

Table 4

Tests of Association of *CD36* Promoter SNP-22674 *T/C* Genotypes with Phenotypic Variables at Baseline, LCD Endpoint and After 6-month Weight-Maintenance Period in Spanish Obese Subjects

Variables	Time point	Genotype 11 Mean ± *SD*	Genotype 12 Mean ± *SD*	Genotype 22 Mean ± *SD*	ANOVA *p*-value	Time *p*-value[b]	Genotype *p*-value[b]
n	68	92	23				
Males/females	32 / 36	46 / 46	13/10				
Age, y	34, 6	35, 6	34, 7				
Weight, kg	Baseline	91.7, 14.4	88.7, 11.6	90.0, 9.9	.43	< .001	.856
	Endpoint	85.0, 13.8	82.5, 11.0	83.0, 9.6	.28		
	6 months	86.0, 13.1	83.1, 11.1	82.9, 10.6	.62		
BMI, kg/m^2	Baseline	31.6, 3.7	30.7, 2.0	30.2, 2.7	.39	< .001	.321
	Endpoint	29.5, 3.8	28.5, 2.1	28.7, 2.6	.20		
	6 months	29.6, 4.1	29.0, 4.1	28.2, 2.7	.43		
Waist circumference, cm	Baseline	91.8, 10.8	92.4, 8.3	94.3, 6.3	.67	< .001	.771
	Endpoint	88.0, 10.8	88.3, 8.2	89.8, 6.5	.24		
	6 months	88.5, 10.4	89.1, 8.6	90.2, 6.3	.50		
Fat mass, kg	Baseline	31.5, 8.5	29.2, 6.3	30.2, 8.2	.17	< .001	.182
	Endpoint	27.4, 7.6	26.3, 6.8	26.0, 6.4	.45		
	6 months	27.6, 7.6	27.2, 6.8	26.1, 6.4	.33		
Fat mass, %	Baseline	34.1, 8.5	33.3, 7.7	32.7, 8.1	.54	.007	.065
	Endpoint	31.3, 8.4	30.8, 7.9	30.5, 9.1	.54		
	6 months	31.6, 8.9	30.7, 8.1	30.5, 7.8	.19		
Cholesterol, mmol/L[a]	Baseline	5.3, 1.0	5.3, 1.1	5.0, 1.0	.15	.001	.036
	Endpoint	4.8, 1.0	4.4, 0.9	4.5, 1.0	.20		
	6 months	4.8, 0.8	4.2, 1.1	3.9, 1.0	.03		
LDL-cholesterol, mmol/l[a]	Baseline	3.5, 1.0	3.4, 0.9	3.1, 0.9	.14	< .001	.030
	Endpoint	3.1, 1.1	2.9, 0.9	2.9, 1.0	.42		
	6 months	3.0, 1.0	2.9, 1.0	2.4, 1.0	.01		
HDL-cholesterol, mmol/l[a]	Baseline	1.4, 0.9	1.4, 1.1	1.4, 0.3	.50	< .001	.107
	Endpoint	1.1, 0.8	1.3, 1.2	1.3, 0.8	.42		
	6 months	1.1, 0.7	1.4, 1.0	1.4, 1.1	.01		
Triglyceride, mmol/l[a]	Baseline	1.2, 1.1	1.2, 1.0	1.1, 0.8	.45	.031	.395
	Endpoint	1.0, 1.2	1.1, 0.9	0.9, 0.9	.90		
	6 months	1.2, 1.1	1.1, 1.0	1.0, 0.4	.80		

Note: [a]Adjusted for BMI

[b]ANOVA for repeated measures for differences among time and between groups based on a co-dominant model.

smaller subset of 654 overweight/obese twins or in 183 similarly overweight/obese Spanish men and women.

However, in the obese/overweight Spanish subjects on a LCD, a –22674 *T/C* genotype effect was observed for total- and LDL-cholesterol during the weight management stages. Moreover, 6 months after the end of the LCD, the *C*-allele carriers showed lower total- and LDL-cholesterol and higher HDL-cholesterol. As expected, the dietary intervention induced a decrease in the SFA intake, but this was more evident in carriers of the *C*-allele. This reduced intake was positively associated with an improved lipid profile (lower LDL- and higher HDL-cholesterol) in the subsequent weight maintenance stage.

The main strength of our study lies in the large size of the twin sample to analyse associations with phenotypes in normal subjects unaffected by perturbations due to disease. Detailed measurements of body fat by DEXA were available in all 2728 subjects. The twin study had 80% (α = 0.05) power to detect a biallelic quantitative trait locus explaining as little as 0.5% of the variance (Purcell et al., 2003). Twins UK subjects have previously been shown to represent the UK female population as a whole (Andrew et al., 2001). The obese and overweight twin subgroup was broadly comparable with the Spanish sample in terms of anthropometric and clinical variables. The main phenotypic differences were in gender (twins were female and the Spanish comprised approximately equal males and females) and average age, (approx. 50 y in twins compared to 35 y in Spanish subjects). Detailed measurements of dietary intake and outcomes in a relatively large sample of Spanish participants enabled examination of nutrigenetic associations with *CD36* variants.

Ma et al. (2004) investigated genetic variability in the *CD36* promoter in relation to plasma NEFA, triglycerides, total- and HDL-cholesterol in 585 non-diabetic Caucasian individuals. They found no associations between –33137 *A/G* and triglycerides, total- or HDL-cholesterol in 217 men or 303 women.

Table 5

Tests of Association of CD36 Promoter SNP -22674 *T/C* Genotype with Dietary Intake at Baseline and at LCD Endpoint in Spanish Obese Subjects

Dietary intake	Time point	Genotype			
		11 (*N* = 68) Mean ± *SD*	12 (*N* = 92) Mean ± *SD*	22 (*N* = 23) Mean ± *SD*	ANOVA *p*-value[a]
Energy, kCal/d	Baseline	2202, 584	2053, 671	2143, 612	0.38
	Endpoint	1461, 442	1382, 278	1430, 381	0.71
Protein, %	Baseline	18.8, 5.1	19, 4.6	19.8, 5.3	0.68
	Endpoint	19.2, 3.3	19.6, 3.4	19.3, 3.0	0.64
Lipid, %	Baseline	39.9, 9.2	39.8, 8.0	41.0, 6.0	0.58
	Endpoint	33.9, 9.2	32.7, 6.5	32.8, 6.6	0.8
Carbohydrate, %	Baseline	40.9, 11.5	41.6, 9.0	39.8, 8.1	0.7
	Endpoint	48.7, 11.7	49.3, 8.6	50.7, 8.5	0.94
Cholesterol, mg/d	Baseline	399, 223	363, 204	427, 240	0.19
	Endpoint	129, 81	144, 169	95, 56	0.31
Fibre, g/d	Baseline	17.5, 8.9	17.8, 10.7	14.8, 7.2	0.52
	Endpoint	21.2, 8.1	19.9, 7.9	22.4, 14.4	0.53
MUFA, g/d	Baseline	43.7, 13.7	42.1, 16.7	44.8, 14.8	0.4
	Endpoint	29.3, 11.7	27.4, 9.3	26.3, 5.5	0.45
PUFA, g/d	Baseline	13.5, 6.1	12.5, 7.4	15.7, 9.6	0.11
	Endpoint	7.3, 3.4	6.5, 2.2	7.3, 7.0	0.22
SFA, g/d	Baseline	28.0, 10.4	25.0, 10.9	26.4, 9.5	0.13
	Endpoint	12.4, 6.9	10.5, 4.4	10.7, 2.9	0.13

Note: [a]*p*-values under co-dominant model.

Our association of –22674 *T/C* with LDL-cholesterol was only evident in 2396 women and not in a smaller sample of 183 Spanish subjects, comparable in size to the gender groups of Ma et al. (2004). We investigated -22674 *T/C* because these authors showed that it is in strong LD with a 5-SNP haplotype associated with higher levels of NEFA and triglycerides than in non-carriers. They did not test associations with this SNP directly. We have no comparable data on NEFA but were able to detect an association with LDL-cholesterol in a much larger group of subjects. In a recent investigation of response to dietary manipulation Madden et al. (2008) tested effects of fish oil on fasting plasma tiglyceride, NEFA, glucose, LDL- and HDL-cholesterol concentrations in relation to the CD26 5-SNP promoter haplotype in 111 healthy Caucasian men. They found that the supplement evoked a significantly greater reduction of serum triglycerides only in carriers of the –33137 *GG* genotype and no associations with lipoproteins or NEFA. The *G*-allele is represented in the 5-SNP haplotype in LD with the –22674 *C*-allele, providing further evidence to support –22674 *C* as a variant modulating CD36 function in response to dietary influence.

Changes in plasma lipoproteins in *CD36*-deficient mice have been observed. In the proximal intestine, CD36 is concentrated on the apical surface of epithelial cells. Drover et al. (2005) found *CD36*-null mice fed a high-fat diet accumulated neutral lipid in the proximal intestine owing to an impaired ability of enterocytes to take up fatty acids and synthesize triglycerides, which in turn led to defective lipoprotein secretion. In the plasma, this was masked by slow clearance of intestine-derived lipoproteins. The impaired clearance may have reflected feedback inhibition of lipoprotein lipase by accumulating fatty acids. The associations with lower LDL-cholesterol that we observed in response to the LCD may devolve from reduced ability of –22674 *CC* homozygotes to take up fatty acids from the intestine, to synthesize triglycerides and secrete LDL-cholesterol.

The pathological effects of CD36 deficiency in humans are currently unclear. A study with a small number of subjects suggests an association with dyslipidemia and impaired insulin action (Miyaoka et al., 2001). It would be of interest to determine whether the putative functional SNP –22674 *T/C* influences *CD36* promoter activity and to examine the link between functional levels of CD36 and lipid profile in humans. SFA tended to be lower in obese carriers of the minor alleles. As *CD36* has been reportedly influential in preference for dietary fats (Laugerette et al., 2005), this preliminary observation would justify a larger study.

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